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# Body composition, volumetric and areal bone parameters in male-to-female transsexual persons

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ABSTRACT

*Context:* Male-to-female (M→F) transsexual persons undergo extreme changes in gonadal hormone concentrations, both by pharmacological and surgical interventions. Given the importance of sex steroids for developing and maintaining bone mass, bone health is a matter of concern in daily management of these patients.
*Objective:* To provide data on bone metabolism, geometry and volumetric bone mineral density in M→F transsexual persons.
*Design/setting/participants:* Twenty-three M→F transsexual persons, recruited from our gender dysphoria clinic and at least 3 yrs after sex reassignment surgery, together with 46 healthy age- and height-matched control men were included in this cross-sectional study.
*Main outcome measures:* Body composition, areal and volumetric bone parameters determined using DXA and peripheral quantitative computed tomography. Hormone levels and markers of bone metabolism assessed using immunoassays. Peak torque of biceps and quadriceps muscles and grip strength assessed using an isokinetic and hand dynamometer, respectively.
*Results:* M→F transsexual persons presented lower total and regional muscle mass and lower muscle strength as compared to controls (all $P<0.001$). In addition, they had higher total and regional fat mass ($P<0.010$) and a lower level of sports-related activity index ($P<0.010$). Bone mineral content and areal density (aBMD) of the lumbar spine, total hip and distal radius, as well as trabecular vBMD of the distal radius was lower as compared to controls ($P<0.010$). At cortical sites, no differences in cortical vBMD were observed, whereas M→F transsexual persons were characterized by smaller cortical bone size at both the radius and tibia ($P<0.010$). Lower levels of biochemical markers of bone formation and resorption ($P<0.010$) suggested decreased bone turnover.
*Conclusion:* M→F transsexual persons have less lean mass and muscle strength, and higher fat mass. In addition, they present lower trabecular vBMD and aBMD at the lumbar spine, total hip and distal radius, and smaller cortical bone size as compared to matched controls. Both the lower level of sports-related physical activity as well testosterone deprivation could contribute to these findings. These results indicate that bone health should be a parameter of interest in the long-term follow-up care for M→F transsexual persons.


## Introduction

The role of sex steroids in pubertal bone development and maintenance of bone mass during aging in men is well established, with both androgens and estrogens as key players in bone metabolism [1,2]. In this regard, the surgical and hormonal treatment of male-to-female (M→F) transsexual persons might have adverse effects on their bone health. The cornerstone for feminisation of males with outspoken gender dysphoria is the administration of exogenous estrogens. As estrogens will be most effective in an environment devoid of androgens, anti-androgen therapy is added to the treatment in some centres, with hormonal therapy generally initiated 2 years before- and estrogens continued after sex reassignment surgery (SRS) [3].

Testosterone (T) deprivation in male patients treated for prostate carcinoma was found to be associated with rapid loss of areal bone mineral density (aBMD) and an increase in bone turnover [4]. However, this was not observed in M→F transsexual persons receiving cross-sex hormonal treatment [5–12]. Moreover, some studies showed an initial increase in lumbar [5,6,9,10] and femoral neck aBMD [9,10] with biochemical indications for a concomitant decrease in bone turnover in the first years during hormonal treatment [5,6]. In

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the longer-term follow-up study by van Kesteren et al. [6], lumbar aBMD returned to baseline values after almost 4 yr of cross-sex hormonal treatment. In addition, Lips et al. [13] performed histomorphometric studies of trans-iliac bone biopsies indicating that anti-androgen and estrogen treatment in M→F transsexual persons was not associated with bone loss and may even suppress bone turnover. An inverse relationship between serum follicle stimulating hormone (FSH) and especially luteinizing hormone (LH) levels and changes in aBMD has been described in both M→F and female-to-male (F→M) transsexual persons [6], though this finding was not replicated in a more recent Norwegian study [12].

From these data, it appears that cross-sex hormonal treatment in M→F transsexual persons does not have deleterious short-term effects on bone metabolism, but may impair bone turnover with limited data on long-term effects. However, most studies cited were performed using dual-energy X-ray absorptiometry (DXA) only and up till now, data on bone geometry and volumetric bone mineral density (vBMD) are lacking. An important limitation of DXA is the reliance on measurement of areal rather than volumetric BMD, with no information of the third-dimension, i.e. the depth of bone. Using peripheral quantitative computed tomography (pQCT), more detailed information on bone mass, volumetric bone density and geometry can be obtained.

This cross-sectional study presents data on volumetric and geometric bone parameters, together with aBMD, body composition, muscle strength and biochemical markers of bone turnover in long-term treated, healthy M→F transsexual persons as compared to age- and height-matched controls.

## Materials and methods

### *Study design and population*

Twenty-three M→F transsexual persons were recruited from our gender dysphoria clinic at the Ghent University Hospital, Belgium. All patients had SRS (orchidectomy and phallectomy in combination with vaginoplasty and usually implantation of breast implants) after 2 yr of hormonal treatment, and at least 3 yr before inclusion in this study. Before surgery, sex reassignment had been initiated using anti-androgen therapy (cyproterone acetate 50–100 mg/day) alone up to a maximum of 1 yr, followed by addition of exogenous estrogen administration (ethinyl estradiol 25–50 μg/day or similar) [14]. Current cross-sex hormonal treatment was not standardized and consisted of: ethinyl estradiol (25–50 μg/day) in 8 participants, estradiol valerate (2 mg/day) in 10 participants, conjugated equine estrogens (1.25 mg/day) in 2 participants and 3 persons were on transdermal estradiol gels. The control population, matched for age and height, consisted of 46 healthy male subjects, recruited from semi-rural communities around Ghent, Belgium. The study protocol was approved by the ethics review board of the Ghent University Hospital (Belgium). All participants gave written informed consent for participation in this study, were in good health and completed questionnaires pertaining to medical history, current and past smoking habits, alcohol consumption, dietary intake of calcium and physical activity during the previous year. Current calcium intake was estimated by a short food questionnaire evaluating weekly averages of dairy products. Physical activity was assessed by recording the weekly frequency of both recreational and/or working activities using Baecke's questionnaire [15]. Alcohol consumption was recorded in units consumed per week. Smoking habits were registered in pack years.

### *Body composition, muscle strength and areal bone mineral density*

Study subjects had their body weight measured to the nearest 0. 1 kg on a calibrated balance scale in light indoor clothing without shoes. Standing height was measured to the nearest 0. 1 cm using a wall-mounted Harpenden stadiometer (Holtain Ltd., Crymuch, UK). Body mass index (BMI) was calculated as weight (kg) divided by height in meters squared ($m^2$). Isokinetic muscle strength (peak torque) of upper arm and leg (biceps and quadriceps muscle, respectively) was assessed at the dominant limbs using an isokinetic dynamometer (Biodex Co., New York, NY, USA). Grip strength at the dominant hand was measured using an adjustable hand-held standard grip device (JAMAR hand dynamometer, Sammons and Preston, Bolingbrook, IL, USA). Their maximum strength was assumed to best reflect the current status and history of their musculoskeletal adaptation, and was expressed in kilograms (kg) and Newton meters (Nm) for grip strength and peak torque, respectively. Whole body soft tissue composition and aBMD at the lumbar spine, proximal femur (total hip region) and the distal forearm (ultra- and mid-distal radius) of the non-dominant limbs was measured using DXA with a Hologic QDR-4500A device (software version 11.2.1; Hologic, Inc., Bedford, MA, USA). *Z*-scores for aBMD were calculated using the age-matched controls provided by the NHANES-III study group for the hip [16] and by the manufacturer for the lumbar spine and distal forearm. Since all subjects underwent normal pubertal development, with known effects on bone size, male reference values were used for all participants. The coefficient of variation (CV%) for both spine and whole body calibration phantoms was <1% as calculated from daily and weekly phantom measurements, respectively.

### *Cross-sectional muscle area and volumetric bone parameters*

All participants had their volumetric bone parameters determined at the dominant forearm and lower leg (midshaft — 66% of bone length from the distal end) by pQCT (XCT2000 scanner, Stratec GmbH, Pforzheim, Germany). At the distal end of the non-dominant forearm (metaphysis — 4% of bone length from distal), a cross-sectional image was obtained to determine trabecular parameters. Single tomographic slices of 2.0 mm thickness were taken at a voxel size of 0.59 mm and 0.80 mm for trabecular and cortical measurements, respectively. Imaging and the calculation of numerical values were performed using the manufacturer's software package (version 5.4). The cross-sectional area (CSA) of the radius/tibia was determined after detecting the outer bone contour at a threshold of 280 mg/$cm^3$. For cortical bone, the threshold was set at 710 mg/$cm^3$. Cortical thickness was estimated using the endosteal and periosteal circumferences. The area moments of inertia (CSMI) were calculated along the latero-lateral, anterior–posterior and polar axes, based on the cortical bone seen in the CT image at the specific threshold. Another index, the dubbed strength strain index (SSI), was proposed by the manufacturer of the pQCT device to reflect bone strength more generally than the CSMI. Latero–lateral, anterior–posterior and polar SSI were calculated. Muscle CSA (CSMA) was estimated using a threshold below water equivalent linear attenuation set at 0.22 $cm^{-1}$. This threshold eliminated skin and fat mass with lower linear attenuation in the cross-sectional slice. From the remaining area bone area was subtracted, revealing the muscle at its maximum CSA. The CV% for the forearm calibration phantom was <1% as calculated from daily phantom measurements.

### *Biochemical determinations*

Venous blood samples and urine specimens were obtained between 08:00 and 10:00 h a.m. after overnight fasting. All samples were stored at −80 °C until batch analysis. Commercial immunoassays were used to determine serum concentrations of C-terminal telopeptides of type I collagen (CTX), pro-collagen 1 aminoterminal propeptide (P1NP), intact parathormone (PTH), LH and FSH (electrochemiluminescence immunoassay; Modular, Roche Diagnostics, Mannheim, Germany; male reference values: 1–9 and 1–12 IU/L, respectively), T and SHBG (Orion Diagnostica, Espoo, Finland), $E_2$ (Clinical Assay, DiaSorin s.r.l., Saluggia,

Italy; according to a modified protocol that doubles the serum amount [17], dehydroepiandrosterone sulfate (DHEAS) and insulin-like growth factor-1 (IGF-1) (Diagnostic Laboratory Systems Inc., Webster, TX, USA), insulin (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden), and leptin (Human Leptin RIA, Linco Research Inc., MO, USA). Serum 25(OH)-vitamin D was determined after extraction by RIA (DiaSorin, Stillwater, MN, USA). Serum free and bioavailable fractions of T and $E_2$ were calculated from serum total T, total $E_2$, SHBG, and albumin concentrations using a previously validated equation derived from the mass action law [18,19]. The intra- and interassay CV% were below 10 and 15% for all measurements, respectively.

### *Statistical analysis*

Continuous variables were described in terms of mean±standard deviation (SD) if their distribution was normal according to the Shapiro–Wilk test, and in terms of median, first and third quartile otherwise. Independent Student *t*-tests or Mann–Whitney-*U*-tests in case of non-Gaussian distribution were used to evaluate differences between cases and controls. When necessary, analysis was done on logarithmically transformed data. Univariate associations were assessed using Pearson's or Spearman's correlation. Linear mixed-effects modelling with random intercepts and a simple residual correlation structure was used to evaluate cross-sectional relationships between muscle strength, physical activity, calcium intake, smoking and volumetric bone parameters in the whole group (variables were chosen based on univariate associations as well as on physiological insights), while adjusting for the confounding effects of age, weight and body height. Parameters of fixed effects were estimated via maximum likelihood estimation. *P*-values<0.05 were considered to indicate statistical significance, all tests were two-tailed. Analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.1.3, Service Pack 4 software (SAS Institute, Inc., Cary, NC, USA).

## Results

### *General characteristics, anthropometry and hormone levels*

General and anthropometric characteristics of the M→F transsexual persons and their age- and height-matched male controls are summarized in Table 1. On average, patients were treated with female steroid hormones for 8 yr, with a minimum of 4 and a maximum of 20 yr, and were 5 yr after SRS (range: 3–17 yr).

**Table 1**
Descriptives of anthropometric and general characteristics

| | M→F transsexual persons (*n*=23) | Healthy control subjects (*n*=46) | *P*-value |
|---|---|---|---|
| Age (yr)[a] | 41±7 | 40±7 | 0.53 |
| Actual smoking (%) | 43.5 | 17.4 | 0.032[b] |
| Ethyl consumption (U/week)[a] | 1.5 [0.8–12.0] | 9.0 [3.0–16.5] | 0.034 |
| Calcium-intake (mg/day)[a] | 528 [431–772] | 544 [423–804] | 0.83 |
| Work activity index | 2.91±0.71 | 2.68±0.79 | 0.30 |
| Sports activity index | 2.28±0.81 | 2.91±0.81 | 0.008 |
| Leisure activity index | 2.49±0.50 | 2.82±0.62 | 0.055 |
| Previous fractures (%) | 34.8 | 39.1 | 0.73[b] |
| Height (cm) | 175±9 | 177±7 | 0.30 |
| Weight (kg) | 75±16 | 80±12 | 0.10 |
| BMI (kg/m$^2$) | 24.4±5.0 | 25.5±3.1 | 0.23 |
| Lean mass (kg) | 51.2±8.4 | 61.8±7.9 | <0.001 |
| Fat mass (kg) | 21.3±7.8 | 16.1±5.8 | 0.008 |
| Grip strength (kg) | 41±8 | 53±8 | <0.001 |
| Biceps peak torque (Nm) | 38±13 | 57±14 | <0.001 |
| Quadriceps peak torque (Nm) | 150±49 | 200±44 | 0.001 |

Data are presented as mean±SD.
[a] Non-Gaussian distribution: data presented as median [1st–3rd quartile].
[b] According to a Chi-square test.

**Table 2**
Descriptives of biochemical and hormonal parameters

| | M→F transsexual persons (*n*=23) | Healthy control subjects (*n*=46) | *P*-value |
|---|---|---|---|
| LH (U/L)[a] | 31.8 [3.8–39.3] | 4.4 [3.1–5.5] | <0.001 |
| FSH (U/L)[a] | 45.4 [8.4–63.6] | 4.3 [2.6–5.7] | <0.001 |
| SHBG (nmol/L)[a] | 106 [67–194] | 30 [19–36] | <0.001 |
| Testosterone (ng/dL) | 32±17 | 590±170 | <0.001 |
| Free testosterone (ng/dL) | 0.3±0.2 | 13.2±3.1 | <0.001 |
| Estradiol (pg/mL)[a,b] | 23.9 [14.2–50.9] | 21.1 [17.1–23.6] | 0.28 |
| Free estradiol (pg/mL)[a,b] | 0.25 [0.16–0.55] | 0.40 [0.32–0.47] | 0.18 |
| DHEAS (μg/dL) | 163±86 | 193±65 | 0.11 |
| Hematocrit (%) | 41.2±2.3 | 45.3±2.3 | <0.001 |
| Creatinine (mg/dL) | 0.78±0.11 | 0.94±0.12 | <0.001 |
| Insulin (IU/L)[a] | 5.5 [3.8–9.9] | 5.6 [4.3–7.2] | 0.99 |
| Glucose (g/dL)[a] | 0.84 [0.79–0.94] | 0.88 [0.83–0.91] | 0.66 |
| IGF-1 (ng/mL) | 210±90 | 280±60 | 0.001 |
| Leptin (ng/mL)[a] | 6.3 [5.5–14.9] | 4.8 [2.5–7.7] | 0.001 |
| 25(OH)vit D (ng/mL)[a] | 23 [14–33] | 18 [13–25] | 0.13 |
| PTH (pg/mL)[a] | 31 [23–39] | 35 [30–45] | 0.09 |
| CTX (ng/mL) | 0.24±0.14 | 0.36±0.16 | 0.003 |
| P1NP (ng/mL)[a] | 32 [24–45] ** | 49 [36–62] | 0.003 |

Data are presented as mean±S.D.
[a] Non-Gaussian distribution: data presented as median [1st–3rd quartile].
[b] Estradiol concentrations only reported for M→F transsexuals persons not taking ethinyl estradiol (*n*=15). ng/dL may be converted to nmol/L by multiplying by 0.0347 for testosterone and pg/mL to by multiplying by 3.676 for estradiol.

In M→F transsexual persons, the percentage of smokers was higher, whereas the weekly consumption of alcoholic beverages was lower. Amongst smokers, the median number of pack year was 15.8 yr, without a significant difference between cases and controls (*P*=0.58). Physical activity during work was comparable, whereas leisure time- and sports-related activity indices were lower in M→F transsexual persons. No differences in fracture prevalence or daily calcium intake were found between cases and controls.

Though not significant, whole body weight tended to be lower in M→F transsexual persons. From DXA-measurements, we found that this resulted from approximately 20% lower total lean mass in M→F transsexual persons, whereas total fat mass was about 30% higher as compared to the control group. In addition, M→F transsexual persons had clearly less muscle strength as compared to healthy controls, both at the upper (grip strength and biceps peak torque) as the lower limb (quadriceps peak torque).

Hormonal and biochemical parameters are listed in Table 2. Serum levels of both gonadotropins, LH and FSH, and of SHBG were higher in M→F transsexual persons, whereas both total and free T serum levels

**Table 3**
Descriptive bone parameters as assessed by DXA at the distal and mid-shaft radius, lumbar spine and total hip

| | | M→F transsexual persons (*n*=23) | Healthy control subjects (*n*=46) | *P*-value |
|---|---|---|---|---|
| Lumbar spine | Total Area (cm$^2$) | 66.7±6.1 | 72.4±6.2 | 0.001 |
| | BMC (g) | 62±13 | 76±12 | <0.001 |
| | BMD (g/cm$^2$) | 0.92±0.14 | 1.05±0.11 | <0.001 |
| | *Z*-score | −1.4±1.3 | −0.3±1.0 | <0.001 |
| Total Hip | Total Area (cm$^2$) | 42.2±5.5 | 45.1±6.7 | 0.024 |
| | BMC (g) | 39±9 | 49±8 | <0.001 |
| | BMD (g/cm$^2$) | 0.91±0.15 | 1.08±0.13 | <0.001 |
| | *Z*-score | −0.6±1.0 | 0.5±0.8 | <0.001 |
| Distal radius | Total Area (cm$^2$) | 4.56±0.41 | 4.67±0.43 | 0.34 |
| | BMC (g) | 1.91±0.39 | 2.29±0.36 | <0.001 |
| | BMD (g/cm$^2$) | 0.42±0.07 | 0.49±0.05 | <0.001 |
| | *Z*-score | −1.8±1.2 | −0.6±1.0 | <0.001 |
| Mid radius | Total Area (cm$^2$) | 9.6±1.7 | 10.3±1.6 | 0.10 |
| | BMC (g) | 6.1±1.2 | 6.8±1.2 | 0.037 |
| | BMD (g/cm$^2$) | 0.63±0.05 | 0.66±0.06 | 0.11 |
| | *Z*-score | −1.1±1.0 | −0.7±1.1 | 0.14 |

Data are presented as mean±S.D.

**Table 4**
Descriptive pQCT measurements of the distal radius (4%), mid-shaft radius and tibia (66%) and cross-sectional muscle and fat area of both limbs

| | Radius | | | Tibia | | |
|---|---|---|---|---|---|---|
| | M→F transsexual persons ($n$=23) | Healthy control subjects ($n$=46) | $P$-value | M→F transsexual persons ($n$=23) | Healthy control subjects ($n$=46) | $P$-value |
| Trabecular bone area ($mm^2$) at 4% | 173±24 | 184±29 | 0.15 | ND | ND | – |
| Trabecular bone density ($mg/cm^3$) at 4% | 180±46 | 227±40 | <0.001 | ND | ND | – |
| Cortical bone area ($mm^2$) | 91±15 | 105±16 | 0.001 | 313±40 | 374±50 | <0.001 |
| Cortical bone density ($mg/cm^3$) | 1101±33 | 1102±33 | 0.87 | 1120±22 | 1108±22 | 0.028 |
| Cortical thickness (mm) | 2.31±0.29 | 2.44±0.29 | 0.084 | 3.93±0.45 | 4.49±0.56 | <0.001 |
| Periosteal circumference (mm) | 46.7±4.5 | 50.7±4.3 | 0.001 | 92.2±7.4 | 97.4±6.5 | 0.004 |
| Endosteal circumference (mm) | 32.2±4.7 | 35.4±4.4 | 0.007 | 67.5±8.1 | 69.2±6.9 | 0.37 |
| $SSI_p$ ($mm^3$) | 350±90 | 420±100 | 0.004 | 2540±530 | 3080±540 | <0.001 |
| Muscle cross-sectional area of the whole limb ($mm^2$) | 3500±700 | 4600±700 | <0.001 | 6600±1300 | 8700±1100 | <0.001 |
| Fat cross-sectional area of the whole limb ($mm^2$) | 1390±530 | 830±320 | <0.001 | 2530±900 | 1600±550 | <0.001 |
| Cortical bone/muscle area[a] | 6.24±1.11 | 5.33±0.48 | <0.001 | 5.91±0.88 | 5.24±0.65 | 0.001 |

Data are presented as mean±S.D. ND=not done.
[a] Calculated as the ratio of total cortical bone area over cross-sectional muscle area, times 100.

were lower. Total nor free $E_2$ levels were significantly different from controls; $E_2$ concentrations are reported only for subjects not taking ethinyl estradiol since this compound is not measured in the estradiol assay. For patients taking ethinyl estradiol, administered doses (25–50 μg/day) were high enough to ensure adequate estradiol exposure. Moreover, for the whole group a clear increase in estrogen exposure in these M→F transsexual persons can be inferred from clinical aspects and from significantly higher serum SHBG levels as compared to controls. Furthermore, no significant differences in serum DHEAS, insulin, glucose, 25(OH)-vitamin D or PTH levels were observed between cases and controls. M→F transsexual persons had lower serum hematocrit, creatinine and IGF-1 levels, whereas they presented higher serum leptin levels. As compared to healthy controls, serum levels of CTX and P1NP, biochemical markers for bone resorption and formation, respectively, were lower in M→F transsexual persons, suggestive for a lower bone turnover in these patients.

*Areal bone densitometry using DXA*

At the lumbar spine, 8 M→F transsexual persons (34.8%) had $Z$-scores below −2, whereas no control subjects were found with $Z$-scores below −2 ($\chi^2$; $P$<0.001). For the total hip, these numbers were 2 (8.7%) and 0 ($\chi^2$; $P$=0.042), respectively. Bone area, bone mineral content (BMC), aBMD and mean $Z$-scores of the lumbar spine, total hip and radius as assessed by DXA are given in Table 3. At the lumbar spine and total hip, markedly lower bone areas, BMC and aBMD were observed in M→F transsexual persons as compared to controls. At the distal radius, with predominantly trabecular bone, BMC and aBMD were lower, with bone area comparable to that of controls. In contrast, at the mid-radius, with predominantly cortical bone, no major differences in bone area or aBMD between M→F transsexual persons and control subjects were observed.

*Cross-sectional muscle (CSMA) and fat area, volumetric bone parameters at the upper and lower limb using pQCT and relations to hormone levels*

Table 4 illustrates pQCT measurements of the distal radius (4%-site), the mid-shaft of radius and tibia (66%-site), and CSMA and fat area of both limbs. In concordance with DXA measurements, we observed clearly lower trabecular vBMD with comparable trabecular bone area at the distal radius in M→F transsexual persons as compared to healthy controls. At cortical sites, no differences in cortical vBMD were found, except for slightly higher cortical vBMD at the tibia in M→F transsexual persons, whereas they presented clearly lower cortical bone area at both the radius and tibia. This resulted mainly from smaller periosteal circumferences as compared to controls. In addition, endosteal circumference at the radius and cortical thickness at the tibia were smaller in M→F transsexual persons as compared to their controls. Consequently, these smaller bones in M→F transsexual persons lead to a lower moment of resistance and SSI in all three directions (only polar SSI shown), and thus less resistance to bending forces as compared to healthy controls.

In line with DXA and muscle strength measurements, M→F transsexual persons presented approximately 25% lower local muscle mass in both limbs, as defined by the CSMA, and approximately 60% higher local fat mass, defined by the cross-sectional fat area. Notably, the cortical bone over muscle area ratio of both limbs was higher in M→F transsexual persons as compared to their controls.

In the M→F transsexual group, we found that neither serum total or free T, $E_2$, SHBG or gonadotropin levels (LH and FSH) were associated with pQCT bone parameters (all $P$>0.180.; data not shown).

*Muscle strength, physical activity, age, smoking and calcium intake in relation to areal and volumetric bone parameters*

Using multivariate analyses to explore contributions of muscle strength, mean level of physical activity, age, smoking and daily calcium intake to areal and volumetric parameters in all participants (both cases and controls; data not shown), muscle strength (grip strength or peak torque) was found to be a major independent predictor of cortical bone size (all $P$<0.014), but not of vBMD, at both the radius and tibia. Further, no major influence of age on bone parameters was found, whereas current smoking was associated with lower cortical vBMD at the tibia ($P$=0.011) and with lower BMC and aBMD at the lumbar spine ($P$=0.026 and $P$=0.004, respectively). Daily calcium intake was weakly negative associated with both peri- and endosteal bone circumference at the tibia ($P$=0.020 and $P$=0.022, respectively). Finally, these analyses showed that mean level of physical activity was associated with cortical BMC and bone area at the tibia ($P$=0.041 and $P$=0.018, respectively).

## Discussion

In this cross-sectional study, we found that long-term treated M→F transsexual persons presented a different body composition, smaller bone size and lower bone turnover as compared to age- and height-matched male controls. In general, M→F transsexuals presented less muscle and more fat mass. Further, markedly lower trabecular vBMD was observed at the distal radius, whereas at sites with predominantly cortical bone (mid-shaft radius and tibia) we observed a significantly smaller bone size, mainly due to a smaller periosteal circumference in these M→F transsexual persons. Cortical vBMD was not different at the radius and even slightly higher at the tibia as compared to the control group. A lower bone strength in these M→F transsexuals

patients is suggested by a smaller cortical bone size, lower aBMD at the lumbar spine, total hip and distal radius and by the relatively high percentage of M→F transsexual participants with *Z*-scores below −2 at the lumbar spine and total hip as compared to controls.

Our results are similar to those of Haraldsen et al. [12], who found that genetic male patients with early-onset gender disorder presented higher fat mass, lower lean mass and aBMD as compared to healthy controls at baseline and prior to any treatment. Despite decreasing levels of bone markers after cross-sex hormonal treatment, no divergent effects on aBMD were found during follow-up, whereas the pre-existing differences in total fat and lean mass increased over time. However, it should be noted that these patients did not receive any anti-androgen treatment and consequently had much higher exposure to T as compared to our patients in the period preceding orchidectomy. Moreover, follow-up aBMD measurements in that study were performed after only 12 months of cross-sex hormonal treatment and before undergoing SRS. Furthermore, our results corroborate with previously published findings: cross-sex hormonal treatment of M→F transsexual persons was associated with a decrease in bone turnover [5,6,12,13] and changes in body composition, namely an increase in fat mass and decrease in muscle mass [20]. However, at variance with our results, most studies did not find a decreased bone mass in M→F transsexual persons receiving cross-sex hormonal therapy [5–12]. In contrast, some studies even reported an increase in lumbar [5,6,9,10] and femoral neck aBMD [9,10] in these patients.

Several mechanisms could underlie our findings. Firstly, near-total deprivation of the anabolic activity of T in these M→F transsexual persons could lead to loss of muscle and gain of fat mass, as has been observed in the study by Elbers et al. in M→F transsexual persons [20] and in men treated for prostate carcinoma [21]. Furthermore, androgens are considered to stimulate periosteal bone expansion [2], which might explain smaller periosteal circumferences at the radius and tibia observed in our M→F transsexual participants. In addition, according to Vanderschueren et al. [2], the higher estrogen exposure in these M→F transsexual persons as compared to healthy controls might even inhibit periosteal bone apposition and/or its interaction with mechanical loading. The importance of sex steroids for periosteal bone apposition is further supported by the histomorphometric analyses by Lips et al. who found that F→M transsexuals presented a larger cortical area as compared to healthy controls after administration of T for 2.5 yr [22].

Secondly, the lower level of sports-related physical activity in M→F transsexual participants could also partially explain the lower muscle mass and strength, and higher fat mass as compared to healthy controls. Moreover, it can be hypothesized that these M→F transsexual persons were already less active during puberty as compared to their peers. This has already been observed in young male patients with early-onset gender identity disorder who avoided competitive physical sports and were less fond of rough and tumble play [23]. It is generally accepted that physical activity during puberty is an important factor contributing to the ultimate bone strength [24]. In this regard, lack of physical activity during growth in these M→F transsexual persons can lead to a less efficient acquisition of peak bone mass and size. Although the slightly higher cortical vBMD found at the tibia in M→F transsexual persons as compared to their controls is most likely due to lower bone turnover in these patients, the observed differences in sports-related physical activity might contribute to this finding. A higher level of physical activity leads to more intra-cortical remodelling, increased cortical porosity and subsequently lower mean material density of cortical bone, as has been observed in post-pubertal males as compared to females [25].

As a result of both deprivation of T exposure as well as the lower level of sports-related physical activity, lower muscle mass and strength lead to lower strains on the bone surfaces in these M→F transsexual persons. From the bone 'mechanostat' theory [26], it is clear that lower muscular strain on bone leads to a lower level of modelling, which mainly promotes periosteal bone apposition, and subsequently to a smaller bone size over time. This is supported by the finding that muscle strength was an important independent positive predictor of bone size at the radius and tibia in our multivariate analyses. In this view, both factors may contribute to the smaller bone size observed in our M→F transsexual participants. Furthermore, the observation of a higher cortical bone/muscle area ratio at both limbs in M→F transsexual persons as compared to their healthy controls might indicate that muscles are more prone to T deprivation and low levels of physical activity, whereas bone size is relatively better preserved. On the other hand, this might also be suggestive for a threshold shift of the 'mechanostat' due to higher estrogen exposure in these M→F transsexual persons, as has been described in pubertal girls and women [27]. This threshold shift would then increase the mechanical sensitivity of the cortical bone, leading to relatively larger cortical bone area under similar muscular strains.

Thirdly, we have to acknowledge the fact that most of our patients were treated with cyproterone acetate alone up to a maximum of 1 yr without concomitant administration of exogenous estrogens [14]. Since cyproterone acetate causes a rapid and substantial decline of serum T levels [28], a decrease in BMD during this first year cannot be excluded. This might possibly explain the variance with previous publications, who found an increase in lumbar [5,6,9,10] and femoral neck aBMD [9,10] under a combination of anti-androgen therapy and administration of exogenous estrogens. Moreover, BMD measurements in the study by Mueller et al. were performed before undergoing SRS [10]. Another possible explanation for the lower aBMD and trabecular vBMD at the distal radius found in our M→F transsexual participants, might be restricted estrogen administration in these patients, as indicated by relatively high levels of gonadotropins in this group. From this and previous studies, it might appear that prevention of bone loss in M→F transsexual persons due to administration of estrogens despite near-total T deprivation only fully succeeds when gonadotropin levels are sufficiently suppressed by adequate estrogen administration. This is supported by the inverse relationship between levels of serum FSH and especially LH levels and changes in aBMD found in the study by van Kesteren et al. [6]. However, no such associations were found in our study or in the study by Haraldsen et al. [12], and the hypothesis of sub-optimal estrogen substitution seems less likely in view of the rather substantial estrogen doses used in most of our patients, reflected by normal serum levels of estradiol in patients not taking ethinyl estradiol, high serum levels of SHBG, clinical aspects and the decreased values for markers of bone turnover. Moreover, our centre has advocated a milder and lower-dose hormonal regimen, in an attempt to avoid short-term complications [29], and from a dose-finding study by Speroff et al., it was found that doses of 5 to 10 μg ethinyl estradiol per day were sufficient to increase lumbar BMD values in postmenopausal women [30].

Finally, the higher percentage of smokers in the M→F transsexual group might provide an additional contribution to their lower aBMD and trabecular vBMD as compared to the control group. From the literature, it is known that smoking is associated with lower bone mass, a higher rate of bone loss and even fracture rate [1], and with reduced cortical thickness at both the radius and tibia in young men [31]. Indeed, we found that current smoking was independently associated with lower BMC and aBMD at the lumbar spine, and with lower cortical vBMD at the tibia. However, no associations between current smoking and bone size were found.

To the best of our knowledge, this is the first study to report on volumetric and geometric bone parameters in long-term treated M→F transsexual persons. These measurements using pQCT provide us with accurate estimates of bone size and vBMD, devoid of the confounding effects of whole body soft tissue composition and bone volume as known from DXA measurements [32]. Furthermore, since all M→F transsexual participants were at least 3 yr post-SRS and on cross-sex

hormonal treatment for at least 4 yr, we can suppose that they were all in a hormonal steady-state and most effects of cross-sex hormonal treatment were established. One of the limitations of this study is its cross-sectional design, which does not allow us to draw any causative conclusions. In addition, since we have no baseline measurements, we cannot exclude that differences in body composition, bone metabolism and size, possibly due to differences in lifestyle, were already present before gender confirming treatment.

In conclusion, we have shown that M→F transsexual persons present lower muscle mass and strength, higher fat mass, lower trabecular vBMD and aBMD at various sites and smaller cortical bone size as compared to healthy age- and height-matched controls. Both the lower level of sports-related physical activity as well as the pharmacological and surgical withdrawal from endogenous T production could contribute to these findings. These data indicate that male-to-female transsexuals may be at increased risk for developing osteoporosis and related fractures. Therefore, bone health should be a parameter of interest in the long-term follow-up care for male-to-female transsexual persons.

## Disclosure information

All authors have nothing to disclose.

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